IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of
Hiromichi KOMORI, et al.
Serial No.: 10/540,521
Filed: June 24, 2005
For: REMEDY FOR DEGENERATIVE INTERVERTEBRAL DISCS

Group Art Unit: 1617
Examiner: Paul Zarek

DECLARATION UNDER 37 C.F.R §1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

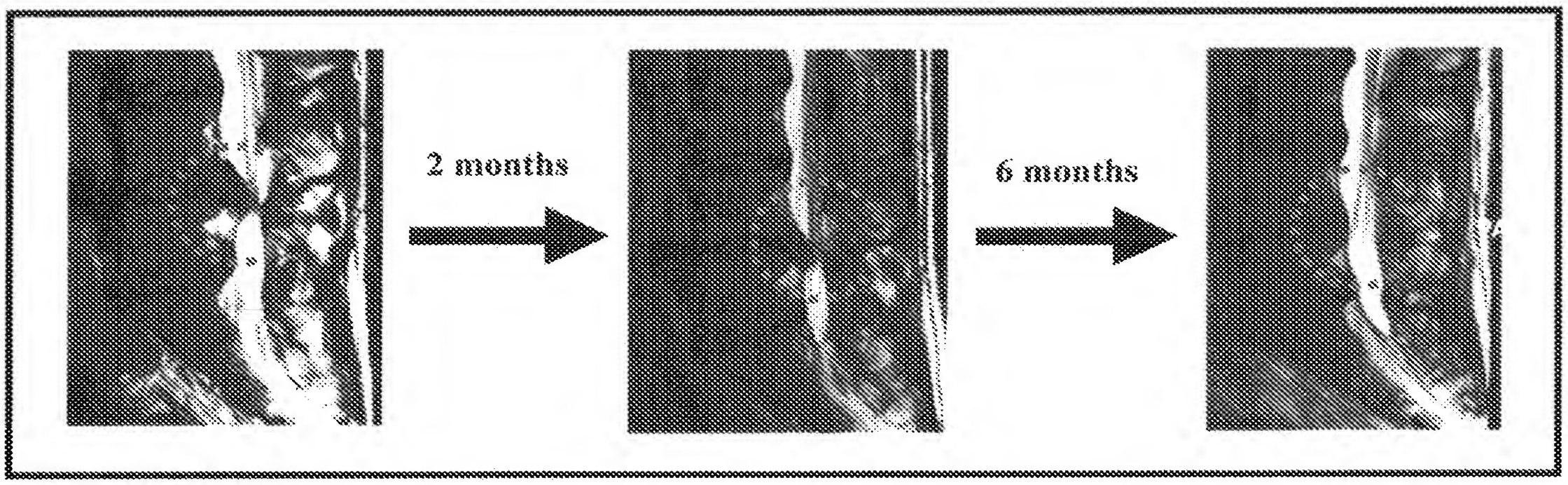
I, Hirotaka Haro, being duly warned, declare that:

1. I am Professor and Chairman of Department of Orthopaedic Surgery, Graduate School of Medicine and Engineering, University of Yamanashi, 1110 Shimokato, Chuo, Yamanashi 409-3898, Japan.
2. My CV is attached demonstrating my qualification to make this declaration.
3. I am fully familiar with the pertinent art of the above-identified U.S. application (hereinafter referred to as "present application" for brevity) as an inventor.
4. I carried out the following explanations and filed experimental evidence to show that the present invention should be patentable.

(1) Technical explanation of "Journal of Clinical Investigation, 2000, p143-150"

I would like to conduct technical explanation of Journal of Clinical Investigation, 2000, p143-150. The treatise was written by me and reported spontaneous resorption mechanism of Herniated disc. The treatise is summarized in Figure 7, and degradation of the disc by the infiltration of macrophages is produced by multi-step interaction between macrophage and chondrocyte. That is, (i) activated macrophages produced TNF-α, which requires the production of MMP-7 to become soluble (sTNF-α), (ii) soluble TNF-α induces chondrocytes to produce MMP-3, (iii) MMP-3 is required for the release of a macrophage chemoattractant and (iv) the subsequent macrophage infiltration of the disc. Macrophage MMP-7 is involved in the spontaneous resorption mechanism of Herniated disc as chemoattractant of soluble TNF-α, which is essential for infiltration of macrophages (also see page 149, left column, paragraph 2). Moreover, it discloses "*in vitro* disc resorption required macrophage infiltration" because it was not sufficient to induce disc resorption when rTNF-α was added directly to wild-type discs in the absence of macrophages on page 147, right column 2 and figure 6.

In addition, because the spontaneous resorption of Herniated disc caused by infiltration of macrophages, it takes a few months before Herniated disc can be resorbed spontaneously.



Thus, this treatise describes spontaneous herniated disc resorption by macrophage infiltration caused by co-expression MMP-3, MMP-7 and sTNF-α in affected region of herniated discs. This treatise does absolutely not describe that herniated discs are degraded by MMP-7 alone without presence of macrophage.

(2) Technical explanation of the present invention

The present invention relates to that herniated disc are degraded by administration of MMP-7 alone without involvement of macrophage. This fact is shown in the Example 1 of the instant specification.

The Example 1 discloses that organ culturing experiment using human surgical herniated disc specimen. In Example 1 human surgical herniated disc specimen were culturing together with MMP-7 alone (sample solution (3)). Thereby, the wet weight of the culture product with MMP-7 was clearly reduced (see fig. 1 and 2).

Thus, by the present invention it is first shown that herniated discs are degraded by administration of MMP-7 alone without involvement of macrophage.

(3) The effect of the present invention

As shown in the Example 3 of the present specification, the nucleus pulposus injected with MMP-7 appears as a degraded matrix, but normal chondrocytes were preserved in the other nucleus pulposus areas and in the annulus fibrosus. On the other hand, in the case using chymopapain in stead of MMP-7, the cartilaginous matrix was degraded throughout the entire nucleus pulposus and annulus fibrosus, with few surviving chondrocytes (Reference Example). Further, in the treatment by chymopapain, immunoreaction and neurotoxicity have been reported (page 4 of the present specification).

In this way, the effect of MMP-7 that the nucleus pulposus injected with MMP-7 appears as a degraded matrix, but normal chondrocytes were preserved in the other nucleus pulposus areas and in the annulus fibrosus, is unexpected and superior for the person skilled in the art of orthopaedic surgery including me. It is believed that the intervertebral disc after administering MMP-7 sustains capacity regenerating the intervertebral disc, because normal chondrocytes are preserved, which have capacity regenerating the intervertebral disc. By extension, it is believed that in prognosis of medical treatment by MMP-7 the intervertebral disc is regenetated normally. This effect is surprising thing, because it is suggested to be difficult to sustain the matrix which is required to support the intervertebral disc regenerative capacity of chondrocytes in the methods directly administering proteases to herniated disc sites for removal of degenerative nucleus pulposus.

(4) The result of the experiment for intradiscal injection into canine discs

Hereinafter, the result of the experiment for intradiscal injection into canine discs is shown.

The histopathology of the intervertebral discs 1 week and 13 weeks after intradiscal administration of MMP-7 was evaluated in male and female beagle dogs (the age of the animals: 9 months old in males and 9-10 months old in females). This study was performed in compliance with Good Laboratory Practice (GLP) Regulations (Ministry of Health and Welfare Ordinance No. 21, 26 March 1997, and as amended, Ministry of Health, Labour and Welfare Ordinance No. 114, 13 June 2008).

Pharmaceutical grade of recombinant human MMP-7 means MMP-7 produced by E. coli expression system, which has 173 amino acids, no disulfide bond and forms a complex with zinc ion. The Molecular formula is $C_{860}H_{1299}N_{239}O_{251}S_4$ and the molecular weight is approximately 19 K dalton.

The animals were anesthetized and a 19-gauge guided needle was inserted down from the epidermis to the intervertebral disc in the lateral decubitus position under X-ray control. When the tip of guided needle reached the annulus fibrosus of the intervertebral disc, a 26-gauge inner needle was inserted into the intervertebral disc itself through the guided needle. Recombinant human MMP-7 at dosing concentrations of 0, 250 and 1000 μg/mL in a dosing volume of 50 μL/disc was injected in 3 intervertebral discs/animal (between second lumber vertebrae and third lumber vertebrae (L2-L3), between third lumber vertebrae and fourth lumber vertebrae (L3-L4), between fourth lumber vertebrae and fifth lumber vertebrae (L4-L5)), and 1 week and 13 weeks after administration necropsy was conducted. Slide specimens of the injection site including vertebral body/bone marrow were stained with Hematoxylin-Eosin (H.E.) and Alcian blue staining, and then were examined microscopically.

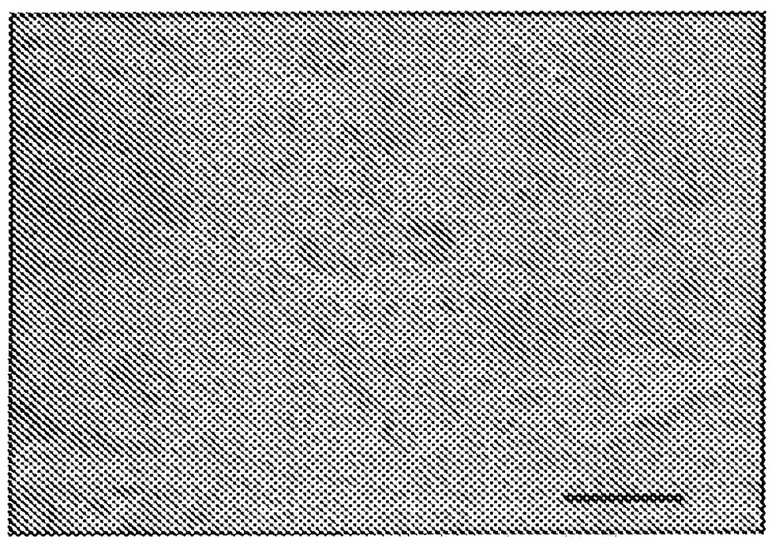
The summary of the result of histopathology of intervertebral discs and surrounding tissues is shown in the below table.

Dose Concentration (μg/mL)	0		250		1000	
Period of Necropsy (Week)	1	13	1	13	1	13
Decrease in nucleus pulposus	-	-	P	P	P	P
Decrease in Alcian blue staining-affinity of intervertebral disc	-	-	P	-	P	-
Macrophage infiltration in intervertebral disc	-	-	-	-	-	-

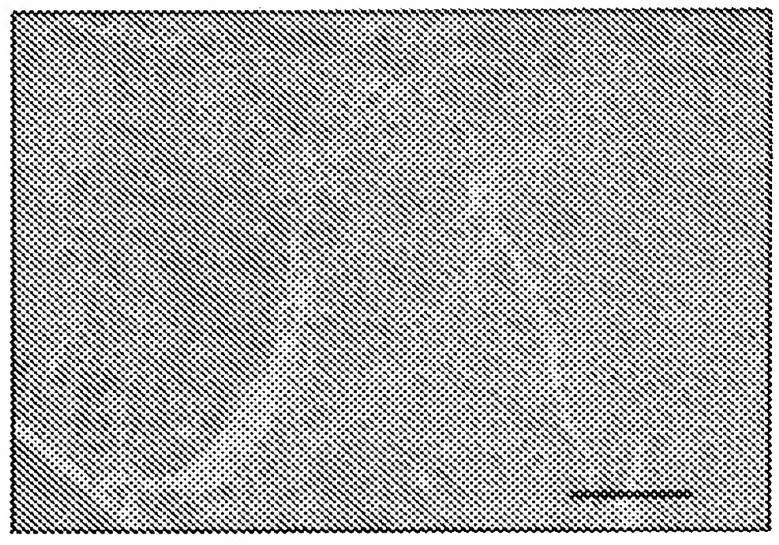
-: No change, P: The change was present

The proteoglycan content of nucleus pulposus, which was determined by the degree of alcian blue staining-affinity on intervertebral discs, can be used as a marker to evaluate the pharmacological effect of MMP-7. In this study, injection of recombinant human MMP-7 in dog intervertebral discs caused the diminished proteoglycan content of nucleus pulposus at 1 week after administration. Decrease in Alcian blue staining-affinity of intervertebral disc was recovered at 13 week after administration.

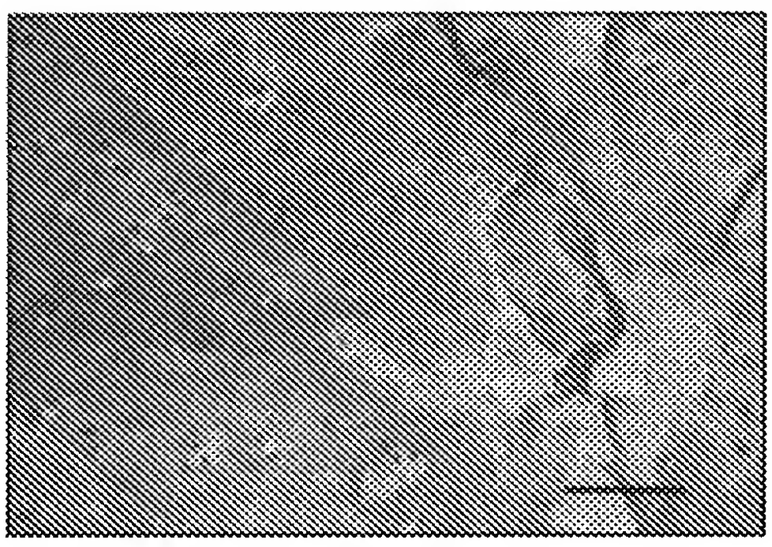
On the other hand, no macrophage infiltration in intervertebral discs were observed in any MMP-7 groups (See below photographs. The bar in photograph represents 100 μm).



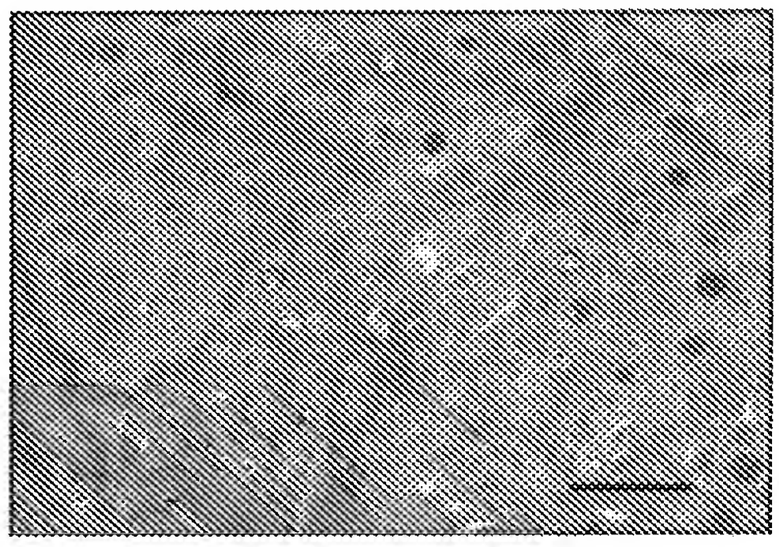
0 μg/mL, 1 week after administration



1000 μg/mL, 1 week after administration



0 μg/mL, 13 week after administration



1000 μg/mL, 13 week after administration

Thus, it is obvious that the proteoglycan in nucleus pulpous is degraded by administration of MMP-7 alone without involvement of macrophage.

For these reasons, I believe that the present invention that directly administrating MMP-7 to the affected site of herniated disc or herniated nucleus pulposus for treating herniated disc or herniated nucleus pulposus should not be considered *prima facie* obvious.

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed this 27th day of April, 2010

Hirotaka Haro

CURRICULUM VITAE

Name: Hirotaka Haro DOB: 1963.4.18. (46 years old)

CURRENT STATUS:

Professor and chairman, Department of Orthopaedic Surgery, Graduate School of Medicine and Engineering, University of Yamanshi.

EDUCATION:

Entered Faculty of Medicine, Yamaguchi University, April 1983, and graduated March, 1989, obtained M.D.

Entered Graduated School of Medicine, Tokyo Medical and Dental University, 1993 and received a Ph.D., January 1997.

LICENSURE & CERTIFICATION:

National Board of Medicine, Registration No. 322870

Japanese Board of Orthopaedic Surgery, Certificate No. 111895

FELLOWSHIP OR STUDY ABROAD:

Visiting Associate Professor at Department of Orthopaedic and Rehabilitation, Vanderbilt University Medical Center, Nashville, TN, USA under Professor Spengler's direction. 1997/April – 1999/ March

MEMBERSHIPS:

Active member of the Japanese Orthopaedic Association

Active member of the Japanese Spine Research Society

Active member of International Society for the Study of the Lumbar Spine (ISSLS)

Active member of Orthopaedic Research Society (ORS)

International active member of American Association of Orthopaedic Surgeons (AAOS)

HONORS & AWARDS:

(1) New Investigator Recognition Award (NIRA) at the Orthopaedic Research Society (ORS) presented at the Annual Meeting in San Francisco, CA, March 2008. Co-authors.

(2) 1st place winner in poster classification: adult spine at the annual meeting of AAOS in Chicago, 2006.

(3) Encouragement Award of the Japanese Orthopaedic Association, 2000.

(4) New Investigator Recognition Award (NIRA) at the Orthopaedic Research Society (ORS) presented at the Annual Meeting in Anaheim, CA, May 1999.

(5) 1st prize Poster Award at the International Society for the Study of the Lumbar Spine (ISSLS) presented at the 22nd Annual Meeting in Helsinki, Finland, June 1995.

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